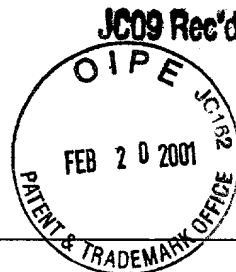


U.S. Application No.
Pending

International Application No.
PCT/BE99/00112



JC09 Rec'd PCT/PTO 20 FEB
09/76353
Attorney Docket No.
VANM198.001APC

Date: February 19, 2001

Page 1

**TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 USC 371**

International Application No.: PCT/BE99/00112
International Filing Date: August 17, 1999
Priority Date Claimed: August 17, 1998
Title of Invention: PHARMACEUTICAL COMPOSITION FOR TREATING OR PREVENTING
DIABETES OR CANCER, OR THE WAARDENBURG SYNDROME
Applicant(s) for DO/EO/US: Guy Rousseau, Frederic Lemaigre

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. (X) This is a **FIRST** submission of items concerning a filing under 35 USC 371.
2. (X) This express request to begin national examination procedures (35 USC 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 USC 371(b) and PCT Articles 22 and 39(1).
3. (X) A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
4. (X) A copy of the International Application as filed (35 USC 371(c)(2))
 - a) () is transmitted herewith (required only if not transmitted by the International Bureau).
 - b) (X) has been transmitted by the International Bureau.
 - c) () is not required, as the application was filed in the United States Receiving Office (RO/US).
5. (X) Amendments to the claims of the International Application under PCT Article 19 (35 USC 371(c)(3))
 - a) () are transmitted herewith (required only if not transmitted by the International Bureau).
 - b) () have been transmitted by the International Bureau.
 - c) () have not been made; however, the time limit for making such amendments has NOT expired.
 - d) (X) have not been made and will not be made.
6. (X) An oath or declaration of the inventor(s) and power of attorney (35 USC 371(c)(4)).
7. (X) A copy of the International Preliminary Examination Report with any annexes thereto, such as any amendments made under PCT Article 34.
8. (X) An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
9. (X) A FIRST preliminary amendment.

U.S. Application No.
Pending

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Date: February 20, 2001

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10. (X) A copy of the International Application as published (minus original claims):
a. (X) Publication Cover Sheet
b. (X) 16 pages of disclosure.
c. (X) 1 page of drawings.
d. (X) Sequence Listing in 11 pages.
e. (X) International Search Report.
11. (X) Small Entity is established.
12. (X) PCT Form PCT/IPEA/402.
13. (X) PCT Form PCT/IB/308.
14. (X) PCT request form.
15. (X) A return prepaid postcard.
16. (X) The following fees are submitted:

				FEES
BASIC FEE				\$860
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total Claims	9 - 20 =	0 ×	\$18	\$0
Independent Claims	1 - 3 =	0 ×	\$80	\$0
TOTAL OF ABOVE CALCULATIONS				\$860
Reduction by 1/2 for filing by small entity (if applicable). Verified Small Entity statement must also be filed. (NOTE 37 CFR 1.9, 1.27, 1.28)				\$430
TOTAL NATIONAL FEE ENCLOSED				\$430

17. (X) A check in the amount of \$430 to cover the above fees is enclosed.
18. (X) A check in the amount of \$40 for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31).
19. (X) The Commissioner is hereby authorized to charge only those additional fees which may be required, now or in the future, to avoid abandonment of the application, or credit any overpayment to Deposit Account No. 11-1410. A duplicate copy of this sheet is enclosed.

U.S. Application No.
Pending

International Application No.
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Date: February 19, 2001

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NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

KNOBBE, MARTENS, OLSON & BEAR, LLP
620 Newport Center Drive
Sixteenth Floor
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Signature

Daniel E. Altman
Printed Name

34,115
Registration Number

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09/763535
JC09 Rec'd PCT/PTO 20 FEB 2001

PCT #

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Rousseau, et al) Group Art Unit Unknown
Int'l Appl. No. : PCT/BE99/00112)
Filed : August 17, 1999)
For : PHARMACEUTICAL)
COMPOSITION FOR)
TREATING OR PREVENTING)
DIABETES OR CANCER, OR)
THE WAARDENBURG)
SYNDROME)
Examiner : Unknown)

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Preliminary to examination on the merits, please amend the above-captioned patent application as follows:

IN THE SPECIFICATION

On page 1, line 4, please insert --RELATED APPLICATIONS

This is the U.S. National Phase under 35 U.S.C. §371 of International Patent Application PCT/BE99/00112, filed August 17, 1999.--.

On page 17, line 1, please cancel the word "CLAIMS" and substitute in its place --WHAT IS CLAIMED IS:--.

IN THE CLAIMS

Please amend the claims as follows:

1. (Amended) A pharmaceutical composition comprising [a suitable pharmaceutical vehicle and] an element [chosen]selected from the group consisting of: a

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Filed : August 17, 1999

[**nucleotide sequence**]polynucleotide encoding a peptide of the ONECUT family, a vector comprising [**this nucleotide sequence**]said polynucleotide, the polypeptide [**sequence**] encoded by [**this nucleotide sequence**]said polynucleotide and[/or] a cell line transformed with said vector [**and expressing the peptide of the ONECUT family**].

2. (Amended) The pharmaceutical composition [**as claimed in**]of claim 1, [**characterized in that**]wherein the protein of the ONECUT family is an isoform of HNF-6 [**in its two isoforms**].

3. (Amended) The pharmaceutical composition [**as claimed in**]of claim 1, [**characterized in that**]wherein the protein of the ONECUT family is OC-2, the amino acid sequence of which is SEQ ID No. 2.

4. (Amended) The [**cellular**] pharmaceutical composition [**as claimed in**]of claim 1, [**characterized in that**]wherein the protein of the ONECUT family is OC-3, the amino acid sequence of which is SEQ ID No 3.

5. (Amended) The pharmaceutical composition [**as claimed in any one of the preceding claims, characterized in that**]of Claim 1, wherein said [**nucleotide and polypeptide sequences are**]polynucleotide is a human polynucleotide[**nucleotide and polypeptide sequence**].

6. (Amended) The pharmaceutical composition [**as claimed in any one of the preceding claims, characterized in that**]of Claim 1, wherein the vector is [**chosen**]selected from the group consisting of plasmids, viruses, phagemids, lipid vesicles, in particular cationic vesicles, liposomes [**or**]and a mixture [**of these**]thereof.

7. (Amended) [**The use of the pharmaceutical composition as claimed in any one of the preceding claims, for preparing a medicinal product intended**]A method for the prevention and/or for the treatment of type 1 or type 2 diabetes or of disorders linked to diabetes, for the prevention and/or for the treatment of cancer[, **in particular of melanoma,**] and for the prevention and for the treatment of Waardenburg syndrome, comprising:

administration of the pharmaceutical composition of Claim 1 in an amount effective to prevent or reduce the symptoms of diabetes, cancer, and/or Waardenburg syndrome.

8. (Amended) [**A**]The method of [**therapeutic treatment of a patient, preferably of a human patient, likely to develop or suffering from diabetes, from a cancer, in particular from a melanoma, or from Waardenburg syndrome, characterized in that**]Claim 7, wherein

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the pharmaceutical composition [as claimed in any one of claims 1 to 4] is administered *ex vivo* by isolating a body fluid or one or more cells from the patient, treating said cells or the cells present in this body fluid with [the]said pharmaceutical composition [of the invention or with the vector included in this pharmaceutical composition], and reinjecting [into said patient] the transformed cells into said patient.

Please add the following claims:

9. The method of Claim 7 wherein the cancer is melanoma.

REMARKS

The Specification and Claims have been amended to correct minor informalities and conform to practice before the United States Patent and Trademark Office. No new matter has been added herewith.

Conclusion

Should there be any questions concerning the above-captioned patent application, the Examiner is respectfully requested to contact the undersigned at the telephone number appearing below.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 20 Feb 2001

By: Daniel Altman
Daniel E. Altman
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Sixteenth Floor
Newport Beach, CA 92660

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CLAIMS

1. A pharmaceutical composition comprising An acceptable pharmaceutical vehicle and an element chosen from the group consisting of a nucleotide sequence
5 encoding a protein of the ONECUT family characterized by the presence of a single CUT domain and the presence of an F48M50 dyad in the homeo domain, a vector comprising this nucleotide sequence, the protein sequence encoded by this nucleotide sequence and/or a
10 cell line transformed with said vector and expressing said protein of the ONECUT family.
2. The pharmaceutical composition as claimed in claim 1, characterized in that the protein of the ONECUT family is HNF-6 in its two isoforms.
- 15 3. The pharmaceutical composition as claimed in claim 1, characterized in that the protein of the ONECUT family is OC-2, the amino acid sequence of which is SEQ ID No. 2.
4. The cellular pharmaceutical composition as
20 claimed in claim 1, characterized in that the protein of the ONECUT family is OC-3, the amino acid sequence of which is SEQ ID No 3.
5. The pharmaceutical composition as claimed in any one of the preceding claims, characterized in that
25 said nucleotide and polypeptide sequences are human nucleotide and polypeptide sequences.
6. The pharmaceutical composition as claimed in any one of the preceding claims, characterized in that the vector is chosen from the group consisting of
30 plasmids, viruses, phagemids, lipid vesicles, in particular cationic vesicles, liposomes or a mixture of these.
7. The use of the pharmaceutical composition as claimed in any one of the preceding claims, for
35 preparing a medicinal product intended for the prevention and/or for the treatment of type 1 or type 2 diabetes or of disorders linked to diabetes, for the

prevention and/or for the treatment of cancer, in particular of melanoma, and for the prevention and for the treatment of Waardenburg syndrome.

8. A method of therapeutic treatment of a patient,
- 5 preferably of a human patient, likely to develop or suffering from diabetes, from a cancer, in particular from a melanoma, or from Waardenburg syndrome, characterized in that the pharmaceutical composition as claimed in any one of claims 1 to 4 is administered ex
- 10 vivo by isolating a body fluid or one or more cells from the patient, treating said cells or the cells present in this body fluid with the pharmaceutical composition of the invention or with the vector included in this pharmaceutical composition, and
- 15 reinjecting into said patient the transformed cells.

PHARMACEUTICAL COMPOSITION INTENDED FOR THE TREATMENT
OR FOR THE PREVENTION OF DIABETES, OF CANCER OR OF
WAARDENBURG SYNDROME

5 Subject of the invention

The present invention relates to a novel
pharmaceutical composition intended for the treatment
or for the prevention of diabetes or of cancer, in
particular to a cellular therapy for diabetes by
10 creating an artificial pancreas.

The present invention also relates to a
diagnostic device intended for the diagnosis and for
the monitoring of the progression of diabetes or of
cancer.

15

Technological background forming the basis of the
invention

Diabetes is a generic term under which are
designated disorders characterized by the combination
20 of polyuria and polydipsia. Diabetes mellitus, also
named hereinafter sugar diabetes, which can be type 1
or type 2 diabetes, is due to poor functioning of the
beta cells of the endocrine pancreas (islets of
Langerhans), which synthesize and secrete insulin
25 (Gerich & Haeften, *COED* 5, pp. 144-148 (1998)). It is
often accompanied (type 2 diabetes) by resistance of
the target tissues to the action of the insulin.

Sugar diabetes is one of the most common
metabolic diseases, in particular in the industrialized
30 world (Leahy, *COED* 5, pp. 73-74 (1998)). It is
characterized by a deficiency of the use of glucose,
and can have serious and sometimes fatal pathological
consequences, such as metabolic disorders,
cardiovascular and neurological problems or retinal or
35 renal lesions. Treatment with insulin requires one or
more daily injections for life.

Consequently, there is a real need to replace
these injections with transplantable systems (Gage et
al., *Nature* 392, Supplement 3 (1998)).

00220-5559/00

State of the art

The document Lemaigre et al. (1996) describes a cDNA encoding hepatocyte nuclear factor 6, named hereinafter HNF-6. The naming of this molecule as hepatocyte nuclear factor (HNF) is an arbitrary naming which indicates that this molecule is a factor which is present in the nuclei of hepatocytes without prejudging whether or not it is related to the other molecules also identified as hepatocyte nuclear factor HNF-1 to HNF-4. This HNF-6 protein controls the transcription of certain genes in a small number of tissues in which it is expressed (Samadani & Costa (1996)). The expression of this molecule has in particular been identified in the mouse pancreas (Landry et al. (1997) and Rausa et al. (1997)).

It is also known that the HNF-6 molecule exerts a control on HNF-4 synthesis in cells in culture. However, none of these documents mentions that modification of the animal or human gene encoding HNF-6 is capable of causing diabetes in a whole organism.

Contrary to what is suggested in document WO 98/11254 and the publication by Duncan et al. (Science Vol. 282, pp. 692-695, July 1998), the HNF-3 molecule controls the synthesis of the HNF-4 molecule in cells in culture, but a modification affecting the gene encoding the HNF-3 molecule is not capable of causing diabetes in animals, including humans.

French patent application FR-2,696,755 describes an implantable capsule comprising an external case consisting of a hydrogel of acrylonitrile and of sodium methallylsulfonate and an internal core comprising an encapsulated substance which can consist of islets of Langerhans, of pancreatic beta cells or of hepatocytes. The case is a biocompatible membrane which is selectively permeable to insulin or to the nutrients required for the substance to be encapsulated. This product can be used in the transplantation of cells or of groups of cells such as islets of Langerhans, in

order to overcome the insufficiency of insulin production in diabetic patients.

International patent application WO 95/09231 describes novel beta-insulin-secreting cell lines which
5 can be in the form of "pseudo-islets" which can be encapsulated in a biocompatible hydrogel, which hydrogel is optionally incorporated into transplantable fibers intended to be introduced into the patient via a subcutaneous or intraperitoneal route in such a way as
10 to treat insulin-dependent diseases.

International patent application WO 95/29988 describes a method for culturing cell lines, in particular pancreatic cells, capable of creating cellular islets which are reimplantable in vivo in
15 mammals in such a way as to treat pancreatic diseases in humans or animals.

An essential characteristic of the cell lines which can be used in substitutive therapy for insulin-dependent diabetes is to be able to secrete insulin in
20 response to glucose (glucose-stimulated insulin secretion, GSIS). This presumes that, in these cells, the expression of the genes involved in GSIS is stable. GSIS depends in particular on GLUT-2, which is the glucose transporter in beta cells, and on glucokinase,
25 which is required for producing the GSIS signal from glucose. A recurrent problem of these lines is the loss of GLUT-2 and of glucokinase (Newgard et al.; (1997)). Another problem is apoptosis (Hohmeier et al., (1998)), cell death which can be caused by glucocorticoid
30 hormones.

Aims of the invention

The present invention is directed toward providing a novel pharmaceutical composition which is
35 capable of being used in the prevention or treatment of diabetes or of cancer and which can be used either in the domain of genetic therapy or the domain of cell therapy in the form of cellular masses or the formation of an artificial pancreatic tissue or organ.

Another aim of the invention consists in providing a novel diagnostic device, such as a diagnostic kit intended to improve the diagnosis and/or monitoring of diabetes or of cancer, in particular to
5 differentiate certain malignant progressions of the cancer.

Characteristic elements of the invention

The inventors have discovered, unexpectedly,
10 that knocking out the HNF-6 gene in mice shows that this gene is essential for the functioning and formation of the islets of Langerhans and for the response of the organism to insulin. In addition, the inventors have shown that other proteins similar to
15 HNF-6, which share with HNF-6 two particularities; on the one hand, the presence of a single cut domain and, on the other hand, the presence of the F48M50 dyad in the homeodomaine (Lannoy et al. (1998)), belonging to the same family named ONECUT (abbreviation OC) (Lannoy
20 et al., (1998) and Jacquemin et al. (1999)), are also involved in some essential metabolic mechanisms. Among the family of proteins thus defined, which comprises in particular the HNF-6 protein, the OC-2 protein and the OC-3 protein, certain proteins have functions which are
25 essential in animals, in particular in humans, especially in glucose metabolism. Mice in which the HNF-6 gene has been knocked out (hnf6-/- mice) have sugar diabetes. This is characterized by a GLUT-2 deficiency in the beta cells and by insufficient
30 insulin secretion in response to glucose (Jacquemin et al., submitted for publication). The diabetes of the hnf6-/- mice is finally cured spontaneously, and this is accompanied by a large increase in OC-2 in the pancreas. These observations illustrate the importance
35 of HNF-6 and of OC-2 in maintaining carbohydrate homeostasis, in particular through maintaining the differentiated phenotype of the beta cells. The inventors have shown, moreover, that HNF-6 can inhibit the effect of glucocorticoids (Pierreux et al. (1999)).

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In addition, such molecules may be used for treating, preventing or diagnosing the appearance and/or development of a certain number of disorders and of diseases, in particular diabetes or cancer, preferably melanoma.

The present invention therefore relates to a pharmaceutical composition comprising a suitable pharmaceutical vehicle and an element chosen from the group consisting of a nucleotide sequence encoding a protein which is a member of the ONECUT family, in particular the HNF-6, OC-2 or OC-3 molecules, the nucleotide and peptide sequences of which are described hereinafter, a vector comprising said nucleotide sequence, the encoded polypeptide sequence and/or a cell line transformed with said vector and expressing these said nucleotide sequences, which are in particular capable of encoding the HNF-6 protein or another member of the ONECUT family, such as the OC-2 or OC-3 molecules.

The expression "nucleotide sequence encoding HNF-6" is intended to mean, for the HNF-6, OC-2 or OC-3 protein, a nucleotide sequence the coding portions (included in the exons) of which correspond, respectively, to the coding sequence of the cDNA as already described by Lemaigre et al. (1996) or to the sequences as described below (encompassing the OC-2 and OC-3 sequences), and the sequences having more than 80%, preferably more than 85%, more particularly more than 90% or more than 95% homology (or sequence identity) with the sequence of the cDNA of the HNF-6 molecule as described by Lemaigre et al. (1996) or equivalent sequences capable of hybridizing with these nucleotide sequences (including the sequences of the OC-2 and OC-3 molecules). This hybridization takes place preferably under conditions which are sufficiently stringent so as to identify the various genomic sequences encoding an amino acid sequence identical or similar to the abovementioned sequences, in particular other sequences specific for other

mammals, having the same function or being involved in the same biochemical mechanism, in particular those in the examples below, but possibly different (in particular due to the redundancy of the genetic code).

5 Stringent hybridization conditions are, in particular, as follows: hybridization at 40°C in 50% of formamide, 5x SSC 20 mM sodium phosphate, pH 6.8, washing in 0.2x SSC at 50°C. Modifications of these conditions can be provided by those skilled in the art as a function of
10 the length and of the GC-nucleotide content in the sequence to be hybridized. Other hybridization conditions are in particular those described by Sambrook et al., §§ 9.47-9.51 in *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, Laboratory
15 Press, Cold Spring Harbor, New York (1989)).

According to the invention, the gene encoding the HNF-6 used concerns genomic sequences encoding both the alpha and beta isoforms of HNF-6, as described by Lannoy et al. (1998).

20 The pharmaceutical composition of the invention can be used to produce genetic and/or cell therapy for a patient likely to develop diabetes or suffering from diabetes, or likely to develop a cancer or suffering from a cancer, in particular from a melanoma. In the
25 domain of genetic therapy, the nucleotide sequence of the invention can be administered to the patient or to cell lines from the patient via ex vivo treatment in naked form using methods well known to the person skilled in the art or via a vector, preferably chosen
30 from the group consisting of plasmids, viruses, phagemids, lipid vesicles such as cationic lipids, liposomes or a mixture of these. The vector will incorporate all the elements required to obtain the expression of the nucleotide sequence according to the
35 invention in the patient, preferably in the specific cell lines to be treated, such as the pancreatic cells involved in insulin synthesis, the hepatic cells involved in insulin response or cells of the epidermis

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or of the dermis which are likely to develop a melanoma.

5 The pharmaceutical composition of the Inventor can also be used in cell therapy by direct injection of the cells using an in vivo or ex vivo method or by forming an artificial cellular aggregate as described in patent applications FR-2,696,755, WO 95/09231 and WO 95/29988. Proliferation of the cells transformed with the nucleotide sequence of the invention or the vector
10 of the invention can be obtained using methods well known to those skilled in the art, in particular those described in patent applications WO 97/49728 and WO 95/29988.

15 The pharmaceutical vehicle according to the invention varies according to the method of administration chosen (intravenous, intramuscular, oral, etc.) and is an excipient well known to those skilled in the art, in the form of tablets, pills, capsules, solutions, syrups, etc. This component
20 optionally comprises adjuvants (in particular a growth hormone) well known to those skilled in the art, so as to induce synergistic effects or to suppress certain specific immune or cellular reactions, or so as to reduce certain unwanted side effects or toxic effects
25 of the active principle or of the vehicle of the invention.

The percentage of active product (nucleotide sequence, amino acid sequence or fragments thereof, vector, cell line, etc.) in the pharmaceutical
30 composition can vary according to very wide ranges which are limited only by the frequency of administration, the tolerance and the level of acceptance of the composition according to the invention by the patient.

35 The present invention also relates to the use of the pharmaceutical composition of the invention for preparing a medicinal product intended for the treatment and/or for the prevention of type 1 or type 2 diabetes and of the disorders linked to diabetes, in

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Another aspect of the present invention relates to the method for treating a patient, in particular a patient likely to develop diabetes, suffering from diabetes, likely to develop a cancer or suffering from a cancer, especially a melanoma, by which the pharmaceutical composition of the invention is administered to said patient using an in vivo or ex vivo treatment method.

Homologous sequences are also defined as sequences which can hybridize with the nucleotide sequences SEQ ID No. 1 and SEQ ID No. 3 encoding the OC-2 and OC-3 molecules. This hybridization takes place preferably under sufficiently stringent conditions, as described above.

A final aspect of the present invention therefore relates to a diagnostic device such as a

diagnostic kit comprising said nucleotide and/or peptide sequences of the OC-2, OC-3 and HNF-6 sequences, and the various reagents intended for the diagnosis and for the monitoring of diseases, in particular of diabetes, of cancer, in particular the diagnosis and monitoring of the progression of melanoma for detection based on the technical methods chosen from the group consisting of in situ hybridization, hybridization and identification with labeled antibodies, in particular by the ELISA or RIA technique, methods of hybridization on a filter, on a solid support, in solution, in sandwich or on a gel by dot blot hybridization or by Northern blot, Southern blot or Western blot hybridization, by labeling with isotopes or without isotopes (such as immunofluorescence or biotin labeling), by the so-called cold probe technique or by genetic amplification (or in particular using PCR, RT-PCR, LCR or CPR amplifications), of double immunodiffusion, of counter-immunoelectrophoresis, of hemagglutination or other techniques well known to those skilled in the art which allow specific identification of nucleotide and/or protein sequences.

The diagnostic device can also comprise elements which allow optional purification of a sample obtained from a human or animal body (such as a physiological liquid), prior treatment of this sample and option preamplification of this sample, as well as diagnosis and quantification of this possible nucleotide or protein sequence and an analysis correlated with the general condition of the animal or human patient treated. These various steps can be carried out manually or using an automatic machine.

The present invention will be described in detail in the nonlimiting examples given below with reference to the appended figures.

Example 1

Detection of melanocyte differentiation.

The function of the melanocytes of the skin, in response to irradiation by UV radiation (Carreira), is to protect the keratinocytes against damage to the DNA induced by UV radiation, via the production of the pigment melanin. More than 70 genes which affect melanocyte development have been identified by genetic analysis, and more than 20 of them have been cloned (Opdecamp (1997)).

A transcription factor (microphthalmia-associated transcription factor (MITF)) is involved in melanocyte differentiation in humans (Tachibana (1996)). It is known that mutations in the MITF gene are associated with Waardenburg syndrome (Tachibana (1994)). It is also known that mutants of the Pax-3 or CREB transcription factors which do not have this transcription activity are associated with the type 1 and type 3 Waardenburg syndromes (Tassabehji). Since the Pax-3 gene encodes an MITF-activating molecule, the identification of other transcriptional factors which affect the MITF gene contribute to improving the diagnosis and monitoring of cancerous diseases and can find applications in the treatment and/or prevention of these diseases, in particular of the Waardenburg syndrome.

Experimental procedure/reverse transcription

A reverse transcription PCR (RT-PCR) is carried out in order to detect the expression of the human mRNAs of the OC-2 and HNF-6 molecules in melanocytes and in various melanoma cells, one microgram of total RNA being reverse [transcribed by] using the murine Moloney leukemia virus reverse transcriptase and other reagents (random hexamers (Life technology Inc.)). The cDNAs of these OC-2 and HNF-6 molecules were amplified by PCR and the specificity of the amplified products was identified by Southern blotting experiments, as described by Jacquemin et al. (1997-1999).

The integrity of the RNA preparations was controlled by amplification of a beta-actin cDNA fragment. The negative controls including the RT-PCR were carried out in the absence of reverse transcriptase.

Melanocyte cell lines

The 397-MEL and 526-MEL cell lines were obtained from the National Cancer Institute KANG.

The LB373-MEL, BB74-MEL and LB1622-MEL cell lines were obtained from the Ludwig Institute of Cancer Research, Brussels, Belgium).

Expression of the genes encoding the OC-2 molecule in melanocytes

The ONECUT proteins are in particular expressed in the cells of human skin. However, the levels of mRNA encoding the OC-2 molecule are particularly high. The expression of mRNA encoding the HNF-6 molecule is low in this tissue (Jacquemin et al. (1999)).

In order to identify a cell type expressing the OC-2 molecule, the inventors carried out an RT-PCR analysis of the RNA of melanocytes and of melanomas.

The PCR products were subjected to analysis by Southern blotting based on the use of radioactive probes.

The results given in Fig. 1 show that it is possible to use the nucleotide sequences of the ONECUT family in order to obtain a differentiated diagnosis of the development of the melanoma.

These results show that only the OC-2 gene is expressed in the melanocytes of the skin.

By contrast, the two genes are highly expressed in various melanoma cell lines. These two genes are expressed at similar levels in the melanoma lines, but the general expression varies depending on the cell lines tested.

Additional assays of transfection of cell lines with plasmid constructs made it possible to demonstrate

that the proximal ONECUT binding site of the MITF promoter is important for the activation of this promoter and that the OC-2 and HNF-6 transcription factors can stimulate the MITF promoter (see fig. 2).

5 Through the discovery that the HNF-6 transcription factor is expressed in melanocytes, it appears that the OC-2 transactivation factor is responsible for the stimulation of the MITF promoter in this type of cell line, and therefore involved in melanocyte development.
10 Given that the HNF-6 gene is expressed at particularly early stages of melanocyte differentiation, and that it is also identified in the melanoma cell lines, it may be considered that HNF-6 is a melanoma cell marker and makes it possible, therefore, to distinguish these
15 cells from already differentiated melanocytes.

Consequently, the genetic sequences of the invention can be advantageously used for improving and adding to the diagnosis and monitoring of various infections and pathologies, in particular of certain
20 types of cancer (such as melanoma), and other syndromes, in particular Waardenburg syndrome, which involves modified expression of the human MITF genes which affect in particular the abnormal development of the melanocytes present in the skin, in the ears and in
25 the eyes.

The gene encoding the OC-2 factor is also a suitable candidate in the domain of genetic therapy for controlling melanocyte development or for treating Waardenburg syndrome.

30

Example 2

Cell therapy of a patient

The operating protocol described below can be applied to patients suffering from various pathologies,
35 in particular patients likely to develop diabetes or suffering from diabetes, or likely to develop or suffering from a cancer, in particular a melanoma, or patients affected by Waardenburg syndrome.

It is clearly understood that the pharmaceutical composition of the invention, which is based on a genetic or cell therapy, can also be combined with treatments based on the use of other gene-regulating systems, in particular based on the use of the Pax-3 or CREB transactivation factors previously described (see fig. 2).

Briefly, the treatment consists in implanting, in a diabetic animal, a line of cells which will have been programmed for GSIS by stable transfection of HNF-6 (or of OC-2). As described above, HNF-6 is, in fact, reputed to maintain in cells the expression of the genes of the differentiated phenotype, in particular GLUT-2 and glucokinase in beta cells, and HNF-6 might oppose the apoptotic effect of glucocorticoids on the implanted cells.

Rats (Wistar males weighing 200-250 g) are made diabetic with a single intravenous injection of streptozotocin (55 mg/kg). After two weeks, to confirm that diabetes has set in, the glucose in the urine is assayed (>15 mM by the Ames "strip" test). These rats received, by intraperitoneal injection, 10 or 50 microspheres (800-900 microns in diameter), each containing 200 000 cells of the "test" line. These microspheres, described by Kessler et al. (1992) (see also French patent application FR-2,696,755), are permeable to insulin, which must be able to exit therefrom, and to the signals of GSIS (such as glucose), which must be able to enter therein. They are impermeable to the agents of rejection by the immune system, but not to glucocorticoids. The "test" line is the RIN 1046-38 line, obtained from a rat insulinoma and cultured according to Clark et al. (1990). Stable transfectants either of HNF-6 or of OC-2 are obtained by electroporating the RIN 1046-38 cells with a plasmid vector comprising a bacterial origin of replication, an ampicillin resistance gene, the DNA complementary to OC-2 or to HNF-6 under the control of the cytomegalovirus promoter/enhancer and a complementary

DNA encoding neomycin phosphotransferase. The complementary DNA encoding neomycin phosphotransferase is cloned 3' of an internal ribosome entry site, itself located 3' of the DNA complementary to HNF-6 or OC-2, such that the cytomegalovirus promoter controls the synthesis of a single bicistronic RNA encoding both HNF-6 or OC-2 and neomycin phosphotransferase. A polyadenylation signal, derived from the SV40 virus and located 3' of the complementary DNA encoding neomycin phosphotransferase, ensures the polyadenylation of the bicistronic RNA. After transfection of the RIN 1046-38 cells according to Clark et al. (1997), the stable transfectants are selected by treatment with geneticin (500 µg/ml) for two weeks.

15 This method can be transposed to insulin-dependent (decompensated type II or type I diabetes) humans as described in Aebischer et al. (1999).

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CLAIMS

1. A pharmaceutical composition comprising a suitable pharmaceutical vehicle and an element chosen from the group consisting of a nucleotide sequence encoding a peptide of the ONECUT family, a vector comprising this nucleotide sequence, the polypeptide sequence encoded by this nucleotide sequence and/or a cell line transformed with said vector and expressing the peptide of the ONECUT family.
2. The pharmaceutical composition as claimed in claim 1, characterized in that the peptide of the family is HNF-6.
3. The pharmaceutical composition as claimed in claim 1, characterized in that the peptide of the family is OC-2.
4. The cellular pharmaceutical composition as claimed in claim 1, characterized in that the peptide of the family is OC-3.
5. The pharmaceutical composition as claimed in any one of the preceding claims, characterized in that said nucleotide and polypeptide sequences are human nucleotide and polypeptide sequences.
6. The pharmaceutical composition as claimed in any one of the preceding claims, characterized in that the vector is chosen from the group consisting of plasmids, viruses, phagemids, lipid vesicles, in particular cationic vesicles, liposomes or a mixture of these.
7. The use of the pharmaceutical composition as claimed in any one of the preceding claims, for preparing a medicinal product intended for the prevention and/or for the treatment of type 1 or type 2 diabetes or of disorders linked to diabetes, for the prevention and/or for the treatment of cancer, in particular of melanoma, and for the prevention and for the treatment of Waardenburg syndrome.
8. A method of therapeutic treatment of a patient, preferably of a human patient, likely to develop or

suffering from diabetes, from a cancer, in particular
from a melanoma, or from Waardenburg syndrome,
characterized in that the pharmaceutical composition as
5 vivo by isolating a body fluid or one or more cells
from the patient, treating said cells or the cells
present in this body fluid with the pharmaceutical
composition of the invention or with the vector
included in this pharmaceutical composition, and
10 reinjecting into said patient the transformed cells.

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T0020"EE00260

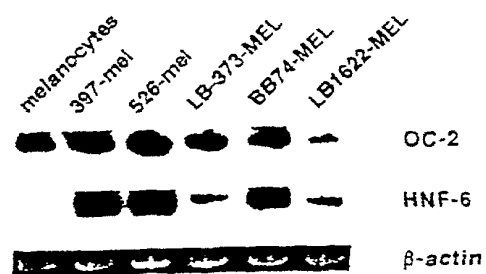


FIG. 1

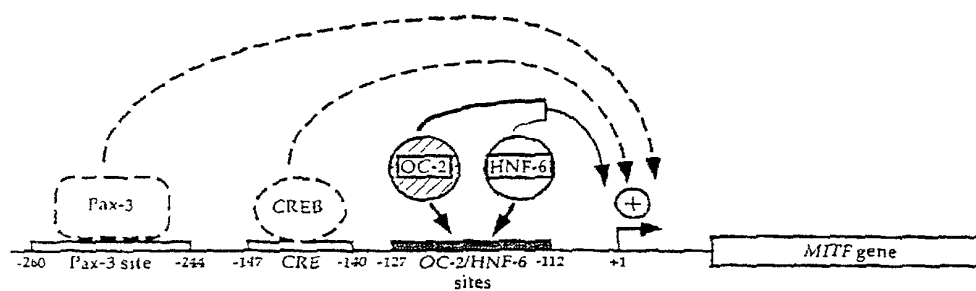


FIG. 2

Declaration and Power of Attorney for Patent Application**Déclaration et Pouvoirs pour demandes de brevet****French Language Declaration**

En tant que l'inventeur nommé ci-après, je déclare par le présent acte que :

Mon domicile, mon adresse postale et ma nationalité figurant ci-dessous à côté de mon nom,

Je crois être le premier inventeur original et unique (si un seul nom est mentionné ci-dessous), ou l'un des premiers co-inventeurs originaux (si plusieurs noms sont mentionnés ci-dessous) du sujet revendiqué, pour lequel une demande de brevet a été déposée concernant l'invention intitulée :

et dont les caractéristiques sont fournies ci-joint à moins que la case suivante n'ait été cochée :

- ☐ a été déposé le
sous le numéro de Demande des Etats-Unis ou
sous le numéro de demande internationale
PCT et modifiée le
(le cas échéant).

Je déclare par le présent acte avoir passé en revue et pris connaissance du contenu des caractéristiques ci-dessus, revendications comprises, telles que modifiées par tout amendement dont il aura été fait référence ci-dessus.

Je reconnais de voir divulguer toute information pertinente à l'examen de cette demande, comme le définit le Titre 37, §1.56 du Code fédéral des réglementations.

As a below named inventor, I hereby declare that :

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled :

**PHARMACEUTICAL COMPOSITION FOR
TREATING OR PREVENTING DIABETES
OR CANCER, OR THE WAARDENBURG
SYNDROME**

the specification of which is attached hereto unless the following box is checked :

- ☒ was filed on
as United States Application Number or PCT
International Application Number
PCT/BE99/00112 filed on **17.08.99** and was
amended on

(if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

French Language Declaration

Je revendique par le présent acte avoir la priorité étrangère, en vertu du Titre 35, § 119 du Code des Etats-Unis, sur toute demande étrangère de brevet ou certificat d'inventeur figurant ci-dessous et ai aussi pris connaissance de toute demande étrangère de brevet ou de tout certificat d'inventeur ayant une date de dépôt précédant celle de la demande à propos de laquelle une priorité est revendiquée.

Prior foreign applications

Demande(s) de brevet antérieure(s)

(Number)	(Country)
(Numéro)	(Pays)
9800609	BE
(Number)	(Country)
(Numéro)	(Pays)
(Number)	(Country)
(Numéro)	(Pays)

Je revendique par le présent acte tout bénéfice, en vertu du Titre 35, § 120 du Code des Etats-Unis, de toute demande de brevet effectuée aux Etats-Unis figurant ci-dessous et, dans la mesure où le sujet de chacune des revendications de cette demande de brevet n'est pas divulgué dans la demande américaine préalable, en vertu des dispositions de premier paragraphe du Titre 35, § 112 du Code des Etats-Unis, je reconnais devoir divulguer toute information pertinente à la demande de brevet comme défini dans le Titre 37, § 1.56 du Code fédéral des réglementations, dont j'ai pu disposer entre la date de dépôt de la première demande et la date de dépôt de la demande nationale ou PCT internationale :

(Application Serial No.)	(Filing date)
(No. de série de la demande)	(Date de dépôt)

(Application Serial No.)	(Filing date)
(No. de série de la demande)	(Date de dépôt)

Je déclare par le présent acte que toute déclaration ci-incluse est, à ma connaissance, véridique et que toute déclaration formulée à partir de renseignements ou de suppositions est tenue pour véridique; et de plus, que toutes ces déclarations ont été formulées en sachant que toute fausse déclaration volontaire ou son équivalent est passible d'une amende ou d'une incarcération, ou des deux, en vertu de la Section 1001 du Titre 18 du Code des Etats-Unis et que de telles déclarations volontairement fausses risquent de compromettre la validité de la demande de brevet ou du brevet délivré à partir de celle-ci.

I hereby claim foreign priority under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

	Priority claimed
	Droit de priorité revendiqué

(Day/Month/Year Filed)	Yes	No
(Jour/Mois/Année de dépôt)	Oui	Non
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(Jour/Mois/Année de dépôt)	Oui	Non
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(Day/Month/Year Filed)	Yes	No
(Jour/Mois/Année de dépôt)	Oui	Non

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application :

(Statut)	(Status)
(Breveté, en attente, annulé)	(Patented, pending, abandoned)

(Statut)	(Status)
(Breveté, en attente, annulé)	(Patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful and false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issued thereon.

French Language Declaration

POUVOIRS : En tant que l'inventeur cité, je désigne par la présente l'(les) avocat(s) et/ou agent(s) suivant(s) pour qu'il(s) poursuive(nt) la procédure de cette demande de brevet et traite(nt) toute affaire avec le Bureau des brevets et marques s'y rapportant.

(mentionner le nom et le numéro d'enregistrement)

POWER OF ATTORNEY : As named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and trademark Office connected there with.

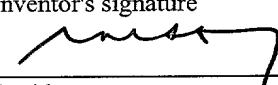
(list name and registration number)

Adresser toute correspondance à :

Send Correspondence to :

Adresser tout appel téléphonique à :
(nom et numéro de téléphone)

Direct Telephone Calls to :
(name and telephone number)

Nom complet de l'unique ou premier inventeur	Full name of sole or first inventor
	<u>ROUSSEAU Guy</u>
Signature de l'inventeur	Inventor's signature
Date	Date
	 24/01/2001
Domicile	Residence
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Nationalité	Citizenship
	Belgian
Adresse postale	Post Office Address
	Avenue de la Chapelle 21 B-1200 BRUSSELS BELGIUM

(Fournir les mêmes renseignements et la signature de tout co-inventeur supplémentaire)

(Supply similar information and signature for any subsequent joint inventor)

Nom complet du second co-inventeur, le cas échéant	Full name of second joint inventor, if any
	<u>LEMAIGRE Frédéric</u>
Signature du second inventeur	Second inventor's signature
Date	Date
	<u><i>Frédéric Lemaigre</i></u> 24/01/2001
Domicile	Residence
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Nationalité	Citizenship
	Belgian
Adresse postale	Post Office Address
	Avenue de l'Ange 30 B-1410 WATERLOO BELGIUM

2nd

Nom complet du troisième co-inventeur, le cas échéant	Full name of third joint inventor, if any
Signature du second inventeur	Third inventor's signature
Date	Date
Domicile	Residence
Nationalité	Citizenship
Adresse postale	Post Office Address

Nom complet du troisième co-inventeur, le cas échéant	Full name of third joint inventor, if any
Signature du second inventeur	Third inventor's signature
Date	Date
Domicile	Residence
Nationalité	Citizenship
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09/763535

528 Rec'd PCT/PC 20 FEB 2001

WO 00/11159

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SEQUENCE LISTING

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<120> PHARMACEUTICAL COMPOSITION INTENDED FOR THE TREATMENT OR
PREVENTION OF DIABETES OR OF CANCER

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09763535-00001

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Ala	Leu	Ala	Ser	Val	Gly	His	Leu	Tyr	Gly	Pro	Tyr	Gly	Lys	Glu	Leu	
					180			185					190			
ccc	gac	atg	ggg	tgc	ccg	ctg	tgc	ccg	ctg	ccc	aac	gag	ctg	ccg	ccc	624
Pro	Ala	Met	Gly	Ser	Pro	Leu	Ser	Pro	Leu	Pro	Asn	Ala	Leu	Pro	Pro	
		195					200				205					
gag	ctg	cac	ggc	gac	ccg	cag	ccc	ccg	ccg	ccg	cca	cca	ccc	ccg	ccg	672
Ala	Leu	His	Gly	Ala	Pro	Gln	Pro	Pro	Pro	Pro	Pro	Pro	Pro	Pro	Pro	
	210					215					220					
ctg	gac	gac	tac	ggc	ccg	cca	ggc	cac	ctg	gac	ggg	gac	aag	ctg	ctg	720
Leu	Ala	Ala	Tyr	Gly	Pro	Pro	Gly	His	Leu	Ala	Gly	Asp	Lys	Leu	Leu	
225					230					235					240	
ccg	ccc	gac	gac	ttc	gag	ccg	cac	gac	ggc	ctg	ctg	gga	cgc	ggc	gag	768
Pro	Pro	Ala	Ala	Phe	Glu	Pro	His	Ala	Ala	Leu	Leu	Gly	Arg	Ala	Glu	
				245					250					255		
gac	gca	ctg	gac	cgc	ggg	ctg	ccc	gga	ggc	ggc	ggc	ggc	aca	ggc	agc	816
Asp	Ala	Leu	Ala	Arg	Gly	Leu	Pro	Gly	Gly	Gly	Gly	Gly	Thr	Gly	Ser	
				260				265					270			
ggc	gga	ggc	ggc	agc	ggg	agc	gac	ggc	ggg	ctg	ctg	ggc	ccg	ctg	ggc	864
Gly	Gly	Ala	Gly	Ser	Gly	Ser	Ala	Ala	Gly	Leu	Leu	Ala	Pro	Leu	Gly	
		275					280					285				
ggg	ctg	ggc	ggc	gac	ggg	ggc	cac	ggg	ccg	cac	ggg	gga	ggc	ggc	ggc	912
Gly	Leu	Ala	Ala	Ala	Gly	Ala	His	Gly	Pro	His	Gly	Gly	Gly	Gly	Gly	
	290					295					300					
ccc	ggc	ggg	agc	ggc	ggc	ggc	ccc	agc	ggc	ggc	gca	ggc	gac	gag	gag	960
Pro	Gly	Gly	Ser	Gly	Gly	Gly	Pro	Ser	Ala	Gly	Ala	Ala	Ala	Glu	Glu	
305					310					315				320		
atc	aac	acc	aag	gag	gtg	ggc	cag	cgc	atc	acc	ggc	gag	ctg	aag	cgc	1008
Ile	Asn	Thr	Lys	Glu	Val	Ala	Gln	Arg	Ile	Thr	Ala	Glu	Leu	Lys	Arg	
				325					330					335		
tac	agc	atc	ccg	cag	gca	atc	ttc	ggc	cag	cgg	atc	ctg	tgt	cgc	tct	1056
Tyr	Ser	Ile	Pro	Gln	Ala	Ile	Phe	Ala	Gln	Arg	Ile	Leu	Cys	Arg	Ser	
			340				345						350			
cag	ggc	acc	ctc	ttc	gac	ctg										

385		390		395		400	
cgc aag gaa cag gag cag cag aag gag cgc gcc ctg cag ccc aag aag							1248
Arg Lys Glu Gln Glu Gln Gln Lys Glu Arg Ala Leu Gln Pro Lys Lys							
	405			410		415	
cag cgc ctg gtg ttc acc gac ctg cag cga cgc acg ctg atc gcc atc							1296
Gln Arg Leu Val Phe Thr Asp Leu Gln Arg Arg Thr Leu Ile Ala Ile							
	420			425		430	
ttc aag gag aac aag cgg ccg tcc aag gag atg cag gtc acc atc tcg							1344
Phe Lys Glu Asn Lys Arg Pro Ser Lys Glu Met Gln Val Thr Ile Ser							
	435			440		445	
cag cag ctc gcc ttc gag ctc aac acc gtc agc aac ttc ttc atg aac							1392
Gln Gln Leu Gly Leu Glu Leu Asn Thr Val Ser Asn Phe Phe Met Asn							
	450			455		460	
ggg cgg cgc cgc tgc atg aac cgc tgg gct gag gag ccc agc acg gcc							1440
Ala Arg Arg Arg Cys Met Asn Arg Trp Ala Glu Glu Pro Ser Thr Ala							
	465			470		475	480
ccc ggg gcc ccc gcc ggc gcc acg gcc act ttc tcc aag gcc tga							1488
Pro Gly Gly Pro Ala Gly Ala Thr Ala Thr Phe Ser Lys Ala							
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Ala Gln Ala Gly Glu Leu Leu Ser Pro Gly His Ala Arg Ser Ala Ala							
	20		25		30		
Ala Gln His Arg Gly Leu Val Ala Pro Gly Arg Pro Gly Leu Val Ala							
	35		40		45		
Gly Met Ala Ser Leu Leu Asp Gly Gly Gly Gly Gly Gly Gly Gly							
	50		55		60		
Ala Gly Gly Ala Gly Gly Ala Gly Ser Ala Gly Gly Gly Ala Asp Phe							
	65		70		75		80
Arg Gly Glu Leu Ala Gly Pro Leu His Pro Ala Met Gly Met Ala Cys							
	85		90		95		
Glu Ala Pro Gly Leu Gly Gly Thr Tyr Thr Thr Leu Thr Pro Leu Gln							
	100		105		110		
His Leu Pro Pro Leu Ala Ala Val Ala Asp Lys Phe His Gln His Ala							
	115		120		125		
Ala Ala Ala Ala Val Ala Gly Ala His Gly Gly His Pro His Ala His							
	130		135		140		
Pro His Pro Ala Ala Ala Pro Pro Pro Pro Pro Pro Pro Gln Arg Leu							

145	150	155	160
Ala Ala Ser Val Ser Gly Ser Phe Thr Leu Met Arg Asp Glu Arg Ala	165	170	175
Ala Leu Ala Ser Val Gly His Leu Tyr Gly Pro Tyr Gly Lys Glu Leu	180	185	190
Pro Ala Met Gly Ser Pro Leu Ser Pro Leu Pro Asn Ala Leu Pro Pro	195	200	205
Ala Leu His Gly Ala Pro Gln Pro Pro Pro Pro Pro Pro Pro Pro	210	215	220
Leu Ala Ala Tyr Gly Pro Pro Gly His Leu Ala Gly Asp Lys Leu Leu	225	230	235
Pro Pro Ala Ala Phe Glu Pro His Ala Ala Leu Leu Gly Arg Ala Glu	245	250	255
Asp Ala Leu Ala Arg Gly Leu Pro Gly Gly Gly Gly Gly Gly Thr Gly Ser	260	265	270
Gly Gly Ala Gly Ser Gly Ser Ala Ala Gly Leu Leu Ala Pro Leu Gly	275	280	285
Gly Leu Ala Ala Ala Gly Ala His Gly Pro His Gly Gly Gly Gly Gly	290	295	300
Pro Gly Gly Ser Gly Gly Gly Pro Ser Ala Gly Ala Ala Ala Glu Glu	305	310	315
Ile Asn Thr Lys Glu Val Ala Gln Arg Ile Thr Ala Glu Leu Lys Arg	325	330	335
Tyr Ser Ile Pro Gln Ala Ile Phe Ala Gln Arg Ile Leu Cys Arg Ser	340	345	350
Gln Gly Thr Leu Ser Asp Leu Leu Arg Asn Pro Lys Pro Trp Ser Lys	355	360	365
Leu Lys Ser Gly Arg Glu Thr Phe Arg Arg Met Trp Lys Trp Leu Gln	370	375	380
Glu Pro Glu Phe Gln Arg Met Ser Ala Leu Arg Leu Ala Ala Cys Lys	385	390	395
Arg Lys Glu Gln Glu Gln Gln Lys Glu Arg Ala Leu Gln Pro Lys Lys	405	410	415
Gln Arg Leu Val Phe Thr Asp Leu Gln Arg Arg Thr Leu Ile Ala Ile	420	425	430
Phe Lys Glu Asn Lys Arg Pro Ser Lys Glu Met Gln Val Thr Ile Ser	435	440	445
Gln Gln Leu Gly Leu Glu Leu Asn Thr Val Ser Asn Phe Phe Met Asn	450	455	460
Ala Arg Arg Arg Cys Met Asn Arg Trp Ala Glu Glu Pro Ser Thr Ala	465	470	475
			480

Pro Gly Gly Pro Ala Gly Ala Thr Ala Thr Phe Ser Lys Ala
485 490

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